Complement protein C1q interacts with DC-SIGN via its globular domain, and thus may interfere with HIV-1 transmission

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Running title: C1q and DC-SIGN in HIV-1 pathogenesis

Abstract

Dendritic Cells (DCs) are the most potent antigen presenting cells capable of priming naïve T-cells. Its C-type lectin receptor, DC-SIGN, regulates a wide range of immune functions. Along with its role in HIV-1 pathogenesis through complement opsonization of the virus, DC-SIGN has recently emerged as an adaptor for complement protein C1q on the surface of immature DCs via a trimeric complex involving gC1qR, a receptor for the globular domain of C1q. Here, we have examined the nature of interaction between C1q and DC-SIGN in terms of domain localization, and implications of C1q-DC-SIGN-gC1qR complex formation on HIV-1 transmission. We first expressed and purified recombinant extracellular domains of DC-SIGN and its homologue SIGN-R as tetramers comprising of the entire extra cellular domain including the α-helical neck region, and monomers comprising of the carbohydrate recognition domain only. Direct binding studies revealed that both DC-SIGN and SIGN-R were able to bind independently to the recombinant globular head modules ghA, ghB and ghC, with ghB being the preferential binder. C1q appeared to interact with DC-SIGN or SIGN-R in a manner similar to IgG. Mutational analysis using single amino acid substitutions within the globular head modules showed that Tyr^{B175} and Lys^{B136} were critical for the C1q-DC-SIGN/SIGN-R interaction. Competitive studies revealed that gC1qR and ghB shared overlapping binding sites on DC-SIGN, implying that HIV-1 transmission by DCs could be modulated due to the interplay of gC1qR-C1q with DC-SIGN. Since C1q, gC1qR and DC-SIGN can individually bind HIV-1, we examined how C1q and gC1qR modulated HIV-1-DC-SIGN interaction in an infection assay. Here, we report, for the first time, that C1q suppressed DC-SIGN-mediated transfer of HIV-1 to activated PBMCs, although the globular head modules did not. The protective effect of C1q was negated by the addition of gC1qR. In fact, gC1qR enhanced DC-SIGN-mediated HIV-1 transfer, suggesting its role in HIV-1 pathogenesis. Our results highlight the consequences of multiple innate immune pattern recognition molecules forming a complex that can modify their functions in a way which may be advantageous for the pathogen.

Introduction

Dendritic Cell Specific Intracellular adhesion Grabbing Non Integrin (DC-SIGN) is a C-type lectin expressed on dendritic cells (DCs) that functions as a pattern recognition receptor (PRR). It can interact with a range of viral, bacterial and fungal pathogens to primarily promote Th2 responses via activation of the mitogen activated protein kinases Erk1 and Erk2 (1) , leading to the clearance of pathogens. DC-SIGN also modulates TLR signaling through activating Serine and Threonine kinase Raf1, which acetylates the NF-κB subunit p65 upon interaction with various pathogens, such as *Mycobacterium tuberculosis, M. leprae, C. albicans,* and measles virus (2, 3) . Acetylation of p65 and increased IL-10 transcription leads to an enhanced anti-inflammatory cytokine response (2) . DC-SIGN also mediates DC-T cell interaction via Intracellular Adhesion Molecule-3 (ICAM-3) (4) . In addition, DCs can adhere to endothelial cells expressing high levels of ICAM-2 via DC-SIGN. Further interactions between Lymphocyte Function-Associated Antigen-1 (LFA-1) and ICAM-1 with ICAM-2–DC-SIGN (5) promote trans-endothelial migration of DCs, allowing them to travel from the blood to the lymphatic system where they can induce T cell responses. Martinez et al have shown that DC-SIGN stimulated CD3-activated T cells produce IL-2, which, in turn, enhances T cell differentiation (6) . DC-SIGN can bind the cell wall component, glycolipid ManLAM of *M. tuberculosis*, and inhibit DC maturation through the suppression of TLR-4 (7) . Such a cross-talk between TLRs and DC-SIGN that generates anti-inflammatory immune response highlights the two faced role of DC-SIGN in immune regulation.

DC-SIGN can bind to HIV-1 envelope protein gp120 through glycan structures (8) and mediate HIV transmission in cis- and trans- fashion. The cis-mode supports DC-SIGN-mediated viral internalization and limited replication; in trans-mode, viral particles are endocytosed and presented to $CD4^+$ cells (9) . DC-SIGN, thus, allows DCs to carry HIV-1 to the lymph nodes where interactions between DCs and T cells leads to transmission of the virus to $CD4^+$ T cells, leading to their infection and eventual depletion (10) . The Hepatitis C Virus (HCV) envelope glycoprotein E2 is another viral protein DC-SIGN engages with (11) . This is achieved through utilizing its high quality endocytic capability to internalize the viral antigen, leading to the infection of DCs (12) .

Structurally, DC-SIGN is composed of an extracellular domain which exists as a tetramer, stabilized by an N-terminal α-helical neck region, followed by a Carbohydrate Recognition Domain (CRD) (8) . Its affinity for N-linked high mannose oligosaccharides is evident through its ligands HIV-1 gp120 and ICAM-3 being highly glycosylated, indicating that this binding is mediated through the CRD region (13, 14) . Studies have shown that the interaction between gp120 and DC-SIGN triggers a drop in IL-6 production by immature DCs. In addition to this, gp120 binding to DC-SIGN has also been shown to suppress the anti-apoptotic activity of Nef and induce apoptosis in immature DCs (14) . Thus, HIV pathogenesis heavily relies on the interplay of molecular mechanisms involving DC-SIGN.

Recently, it has emerged that DC-SIGN interacts with the complement classical pathway recognition protein, C1q (15) , in conjunction with its globular head receptor, gC1qR, on the surface of immature DCs. C1q as well as gC1qR are known to associate with the viral envelope protein gp41 of HIV-1 (16, 17) . C1q has been shown to interact with gp41 ectodomain via its globular head (gC1q) domain (18) , specifically via the A chain (19) , in a way similar to C1q-IgG interaction (20, 21) . Similar to IgG, the ability of gp41 to form aggregates (16) leads to an enhanced activation of the C1 complex, as well as the function of gp41 to initiate the classical pathway on the surface of infected cells in an antibody-independent manner (22) . The C1q binding site on gp41 resides within residues 601-613 of the immunodominant loop region (16) , which contains hydrophobic side chains and forms a cleft (23) .

gC1qR on its own has been shown to suppress the production of HIV-1 in MT-4 and H9 human T cell lines, and macrophages infected with HIV-1_{IIIB} and HIV-1_{Ba-L} (24) Suppression of virus production is further enhanced when gC1qR is pre-incubated with the target cell lines prior to HIV-1 challenge, suggesting that interference with viral entry by gC1qR occurs through interaction with CD4 (24) . gC1qR is known to bind to a range of viral ligands including the HCV core protein (25) , Adenovirus core protein V (26) EBNA-1 (27) and Rubella virus capsid protein (28) . gC1qR can also act as a receptor for HIV-1 gp41 and target healthy $CD4^+$ T cells to Natural Killer (NK) cell-mediated lysis (17) . This bystander effect of autologous killing occurs through the surface translocation of NKP44L, through activation of PI3K, NADPH oxidase and p190 RhoGAP.

Recently, DC-SIGN, C1q and gC1qR on immature DCs have been shown to form a tripartite complex, with a plausible role in DC differentiation through signaling via the NF-κB pathway (15) . Given that each of these innate immune proteins (C1q, DC-SIGN and gC1qR) can bind HIV-1, we set out to dissect the nature of interaction between C1q and DC-SIGN, and examine how it can impact upon HIV-1 transmission.

MATERIALS AND METHODS

Expression and purification of soluble DC-SIGN and SIGN-R

The pT5T constructs expressing tetrameric and monomeric forms of DC-SIGN and SIGN-R were transformed into *Escherichia coli* BL21 (λDE3) (8) . Protein expression was performed in bacterial culture using Luria-Bertani medium containing 50 μg/ml of ampicillin at 37°C until OD_{600} reached 0.7. The bacterial culture was induced with 10mM isopropyl-β-D-thiogalactoside (IPTG) and incubated for a further 3 h. Bacterial cells (1 litres) were centrifuged at 4500 x g for 15 min at 4°C and cell pellet was treated with 22 ml of lysis buffer containing 100 mM Tris, pH 7.5, 0.5 M NaCl, lysozyme (50μg/ml), 2.5 mM EDTA, pH 8.0 and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and left to stir for 1 hour at 4°C. Cells were then sonicated for 10 cycles for 30 seconds with 2 minute intervals and the sonicated suspension was spun at 10,000g for 15 min at 4°C. The inclusion bodies, present in the pellet, were solubilized in 20 ml of 6 M Urea, 10 mM Tris-HCl, pH 7.0 and 0.01% β-mercaptoethanol by rotating on a shaker for 1 h at 4°C. The mixture was then centrifuged at 13,000 x g for 30 min at 4°C and the supernatant was drop-wise diluted 5-fold with loading buffer containing 25 mM Tris-HCl pH 7.8, 1 M NaCl, and 2.5 mM $CaCl_2$ with gentle stirring. This was then dialysed against 2 litres of loading buffer with 3 buffer changes every 3 h. Following further centrifugation at 13,000 x g for 15 min at 4°C, the supernatant was loaded onto a Mannan agarose column (5ml; Sigma) pre-equilibrated with the loading buffer. The column was washed with 5 bed volumes of the loading buffer and the bound protein was eluted in 1 ml fractions using the elution buffer containing 25 mM Tris-HCl pH 7.8, 1 M NaCl, and 2.5 mM EDTA. The absorbance was read at 280 nm and the peak fractions were frozen at -20. Purity of protein was analyzed by 15% w/v SDS-PAGE.

Expression and purification of recombinant wild type globular head modules ghA, ghB and ghC of human C1q, and their substitution mutants

The recombinant globular head regions of human C1q, ghA, ghB, and ghC modules (19) and their respective mutants (29) were expressed in *E. coli* BL21 as fusion to maltose-binding protein (MBP). Bacterial cells were grown in 200 ml LB medium containing ampicillin (100µg/ml) at 37°C, were induced with 0.4 mM IPTG at OD_{600} of 0.6 for 3 h and then centrifuged (4500 x g for 15 min). The cell pellet was suspended in 25 ml of lysis buffer (20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 1 mM EDTA, 0.2% v/v Tween 20, 5% glycerol, 0.1 mM PMSF and 0.1 mg lysozyme) and incubated at 4°C for 1 h on a rotary shaker. Cell suspension was then sonicated for 30 seconds with 2 minute gaps for 10 cycles. After centrifugation (13,000 x g for 15 min), the supernatant was diluted 5-fold in buffer I (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.2% Tween 20, 1 mM EDTA and 5% glycerol), passed through an amylose resin column (15 ml; New England Biolabs), and then washed with 3 bed volumes of buffer I followed by buffer II (50 ml of buffer I without Tween 20). The protein was then eluted in 1ml fractions with 10 mM maltose in 100 ml of buffer II and frozen at -20°C after determining protein concentration and purity via Nanodrop and 10% w/v SDS-PAGE, respectively.

Purification of human C1q from plasma

C1q was purified from freshly-thawed plasma as described previously (19) . Briefly, plasma was made 5mM EDTA, pH 7.5 and centrifuged to remove aggregated lipids. It was then incubated with non-immune IgG coupled to CNBr-activated Sepharose (GE Healthcare, UK) for 1 h at 4°C. The plasma with IgG-Sepharose was filtered through a sintered glass funnel, and C1q-bound Sepharose was then washed extensively with 10 mM HEPES, 140 mM NaCl, 0.5 mM EDTA**,** pH 7.0. C1q was eluted with CAPS (N-cyclohexyl-3-aminopropanesulfonic acid) buffer (100 mM CAPS, 1 M NaCl, 0.5 mM EDTA, pH 11). The eluted C1q was then passed through a HiTrap Protein G column (PierceNet, USA) to remove IgG contaminants and dialyzed against the washing buffer.

Expression and purification of human gC1qR

The recombinant mature gC1qR protein containing 74-282 residues was expressed in *E. coli* BL21 (λDE3) (Pednekar et al, Frontiers in Immunology, in revision). Bacterial cells were grown in 250 ml of LB at 37°C until an OD of 0.6 was reached and induced with 0.5mM IPTG. After 3 h, the bacterial cell culture was spun down (4500g 15min). The cell pellet was treated with lysis buffer (20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 1 mM EDTA, 0.2% Tween, 5% glycerol, 0.1mg lysozyme) and incubated for 1 hour at 4°C with shaking. The cell lysate was then sonicated for 10 cycles at 30 seconds with 2 minute intervals. The lysate was spun down at 13000g for 15min and the supernatant was collected and dialyzed for 2 h against 20 mM Tris-HCl, pH 7.5. The dialyzed protein was subjected to ion exchange using a DEAE column and eluted at a peak of 0.45 M NaCl.

Direct Binding ELISA

Microtitre wells were coated overnight at 4°C with DC-SIGN or SIGN-R (5, 2.5, 1.25 and 0.625 µg/well) in carbonate-bicarbonate buffer, pH 9.6) and left overnight at 4°C. Wells were blocked with 100µl of 2% w/v BSA in PBS for 2 h at 37°C. Following 3 washes with PBS + 0.05% Tween 20, ghA, ghB, ghC, or its substitution mutants (2.5 µg/100 µl) was added to each well in the buffer containing 50 mM NaCl, 100 mM Tris-HCl, pH 7.5 and 5 mM $CaCl_2$. MBP (Sigma) was used as a negative control. The plate was incubated at 37°C for 1.5 h and then at 4°C for 1.5 h. The wells were washed and the bound protein was detected with anti-MBP monoclonal antibodies in PBS (1:5000, Sigma) followed by rabbit anti-mouse IgG-HRP conjugate (1:5000; Sigma) for 1 h. The colour was developed using OPD (o-phenylenediamine dihydrochloride, Sigma) and read at 415 nm.

Competitive ELISA

DC-SIGN and SIGN-R were coated on microtitre wells by overnight incubation at 4°C using 5 µg/well (in 100 µl) in carbonate-bicarbonate buffer pH 9.6. Wells were blocked with 2% BSA in PBS for 2 h at 37°C. Following washing with PBS + 0.05% Tween, the plate was incubated with a steady concentration (5 µg/well) of one competing protein (gC1qR) and various concentrations (5, 2.5, 1.25, 0.625 µg/well) of the second competing protein (ghB) in calcium buffer to give a total of 100 µl per well. After incubating for 1.5 h at 37°C and 1.5 h at 4°C the wells were

washed and anti-gC1qR polyclonal antibody (1:1000) in PBS was added and incubated for a further 1 h at 37°C. Bound protein was detected by Protein A-HRP conjugate (1:5000) and the colour was developed using o-Phenylenediamine dihydrochloride (OPD). Data was plotted to determine inhibition values of competitive ligand binding.

In order to examine if C1q and globular head modules can inhibit binding of DC-SIGN to gp120, microtitre wells were coated with 250 ng of gp120 (Abcam) in carbonate-bicarbonate buffer and left overnight at 4ºC. Plate was blocked with 2% w/v BSA in PBS for 2 h at 37°C, followed by washing three times with PBS + 0.05% Tween 20. Various concentration of ghA, ghB, ghC and C1q (10, 5, 2.5, 0 µg/ml) were co-mixed with 2.5µg/ml of DC-SIGN, and added to wells in calcium buffer (100 µl per well). After incubation for 1 h at 37°C and 1 h at 4°C, the wells were washed again three times using PBS + 0.05% Tween 20. The binding of DC-SIGN to gp120 in the presence of globular heads and C1q as detected using rabbit anti-DC-SIGN antibody (1:500), and probed with protein A-HRP conjugate (1:5000). The colour was developed using 3,3′,5,5′-Tetramethylbenzidine (TMB) and read at 450nm spectrophotometrically.

Western Blotting

Recombinant ghA, ghB and ghC modules (15 µg), in addition to MBP and BSA as negative control proteins, were run separately on a 12% SDS-PAGE gel and transferred onto PDVF membrane for 1 h at 320 mA. Membrane was blocked in 5% non-fat milk (1 h at room temperature) and 50µg of recombinant DC-SIGN (tetramer) in loading buffer (25 mM Tris-HCl, pH 7.8, 1 M NaCl, 2.5 mM $CaCl_2$) was added and incubated overnight at room temperature. The blot was washed three times for 10 min each in PBS containing 0.05% Tween 20 and then incubated with anti-DC-SIGN (1:1000) polyclonal antibody (ProSci) in 1% non-fat milk (2 h at 37°C). Following subsequent washes, the membrane was incubated with Protein A-conjugated HRP (1:1000) (1h at room temperature). The blot was developed using 3, 3′-Diaminobenzidine (Sigma D7679) (DAB) as a substrate.

Fluorescent microscopy

Binding of C1q globular head modules to DC-SIGN expressed on HEK cells

DC-SIGN expressing HEK 293 (DC-HEK) cells, as reported by Lang et al (30) were grown in DMEM-F12 (Life technologies, UK) containing 10% v/v FCS and blasticidin (5µg/ml) (Gibco). The cells were grown on 13mm glass cover slips till a monolayer of cells was formed, and then incubated with 15µg/ml of recombinant ghA, ghB and ghC (MBP as a negative control) separately in serum free medium and left to incubate for 30 min in 37°C. Cells were washed with PBS and fixed using 4% v/v Paraformaldehyde (PFA) for 10 min, rinsed again with PBS three times, and then blocked with 5% FCS for 30 min. The slides were incubated for 30 min with mouse anti-MBP antibody to detect MBP fusion proteins and rabbit anti-DC-SIGN antibody to reveal expression of DC-SIGN in DC-HEK cells. After three washes for 30 min each and incubation with secondary antibodies: Alexa Fluor 568 conjugated goat-anti-mouse antibody (Thermo Fisher) and Alexa Fluor 488 conjugated goat anti-rabbit antibody (Abcam) for 30 min, the slides were then washed in PBS, mounted and observed under Leica DM4000 Fluorescent microscope using Leica Application Suite.

HIV-1 transfer assay with DC-HEK cells and PBMC

Pooled Peripheral Blood Mononuclear Cells (PBMCs) (HiMedia Laboratories, India) were cultured in RPMI 1640 medium (Sigma Aldrich) containing 10% FBS, 1% Penicillin-Streptomycin (Complete RPMI medium), and stimulated with 5 µg/ml phytohemaglutinin (PHA) and 10 U/ml of recombinant-human IL-2 (Gibco) for 24 h. PHA/IL-2 was washed off and activated PBMCs were cultured further for 3 days in complete RPMI 1640 medium.

DC-HEK cells were grown and maintained in DMEM-F12 (Sigma Aldrich USA) containing 10% FBS and blasticidin (5µg/ml) (Gibco). Cells were sub-cultured every 3 days and those in the log phase were used for assays. DC-HEK cells were grown in a 12 well plate until 80% confluence. Indicated concentrations of C1q, ghA, ghB, ghC, gC1qR individually, or in combination, in medium containing 5mM $CaCl_2$ were added to each well and incubated for 2 h to allow binding. Excess protein was removed and cells were challenged with 5 ng/ml p24 of HIV-1 SF-162 strain for 1 h. MBP was added along with the virus as a negative protein control. Unbound virus was washed off and cells were co-cultured with PHA/IL-2 activated PBMCs for

24 h to facilitate viral transfer. PBMCs in the supernatant were then separated from the adhered DC-HEK monolayer and were cultured further for 7 days and viral titre was determined using HIV-1 p24 antigen ELISA of supernatants collected on day 4 and 7 (XpressBio Life Science Products, Frederick, MD). That the reduction in p24 levels was not due to cellular death was confirmed by MTT assay of cultured PBMCs on day 7.

Statistical analysis

Viral transfer experiment data is plotted using Graph-Pad Prism version 5.0 and data has been analysed for statistical significance using one way ANOVA. $P<0.05$ was considered as statistically significant.

RESULTS

Both DC-SIGN and SIGN-R bind to C1q

DC-SIGN and SIGN-R comprising of the entire extracellular domain (ECD) (Figure 1a, c) and the CRD region alone (Figure 1b, d) alone were expressed in *E. coli* and affinity-purified on Mannose-agarose. The CRD regions of DC-SIGN and SIGN-R bound mannose weakly as majority of the proteins appeared in the flow through. The ECD domains of both DC-SIGN and SIGN-R bound to mannose with much greater affinity in the presence of Ca^{2+} and eluted with EDTA. Previously, Kang et al have shown that SIGN-R interacts with C1q (31) . Recently, work by Hosszu et al revealed that DC-SIGN bound directly to C1q (15) . Thus, we examined direct binding of both the tetrameric and monomeric variants of DC-SIGN and SIGN-R with purified human C1q on microtitre plates. Both DC-SIGN (Figure 2a) and SIGN-R (Figure 3a) in their tetrameric and monomeric forms were able to bind to C1q in a dose-dependent manner. Experiment showed a strong binding of the tetramers to C1q when compared to the CRD region alone, with the ability of C1q to bind nearly 50% more when the α-helical neck was intact. C1q bound to SIGN-R (Figure 3a) better than DC-SIGN (Figure 2a). The ability of the globular head modules to bind DC-SIGN was also examined via a far-western blot (Figure 2d), where ghA, ghB and ghC, run on a SDS-PAGE and transferred on a nitrocellulose membrane, were probed with soluble DC-SIGN tetramer. ghA and ghB appeared to bind DC-SIGN well compared to the ghC module. MBP and BSA, used a negative control proteins, did not bind DC-SIGN tetramer.

DC-SIGN and SIGN-R neck region is required for efficient binding to C1q and individual globular head modules

Tetrameric (Figure 2b) and monomeric (Figure 2c) DC-SIGN and SIGN-R (Figure 3b, c) coated on microtitre wells were probed with ghA, ghB and ghC to examine whether these globular head modules were able to bind to the extracellular domain and carbohydrate recognition domain (CRD) with similar avidity. ghA, ghB and ghC bound with much greater affinity to DC-SIGN and SIGN-R tetramer in comparison to the monomers, indicating that the neck is required for the individual globular heads to bind efficiently. Like C1q, the ghA, ghB and ghC modules bound SIGN-R better than DC-SIGN (Figure 3b).

DC-SIGN and SIGN-R bind preferentially to ghB module

Since C1q bound to DC-SIGN and SIGN-R via its globular head region, as evident from the use of ghA, ghB and ghC models, we sought to map their specificity for DC-SIGN and DC-SIGN-R binding (Figure 2b, 3b). When DC-SIGN and SIGN-R were coated on microtitre wells and probed with ghA, ghB and ghC, all three globular heads bounds DC-SIGN and SIGN-R in a dose dependent manner indicating that all three heads are capable of binding to the ligands independently. Furthermore, ghB module was preferential in binding to DC-SIGN (Figure 2b); it bound much better to DC-SIGN compared to ghA and ghC. In addition, ghB was a better binder of SIGN-R, as was ghA for SIGN-R (Figure 3b) than DC-SIGN (Figure 2b), compared with the ghC module. Although binding of ghA, ghB and ghC to the CRD domain only was significantly lower, the ghB module was still a better binder of DC-SIGN (Figure 2c) and SIGN-R (Figure 3c) monomers.

The ghA substitution mutants bind differentially to DC-SIGN and SIGN-R

The ability of substitution mutants $Arg^{A162}Glu$ and $Arg^{A162}Ala$ to bind to DC-SIGN and SIGN-R was assessed by ELISA. Both substitution mutants bound DC-SIGN (Figure 4a) and SIGN-R (Figure 5a) in a dose-dependent manner. SIGN-R was able to interact with $Arg^{A162}Glu$ nearly as efficiently as it did with the wild-type ghA, showing a reduction in binding of only 15% (5%) at the highest concentration of 5µg (Figure 5a). $Arg^{A162}Ala$, on the other hand, bound SIGN-R with much less affinity, showing a drop of 27% (Figure 5a). Considering DC-SIGN and SIGN-R are both highly conserved, $Arg^{A162}Glu$ was able to interact with DC-SIGN weakly than it did with SIGN-R (Figure 4a, 5a), showing a ~35% reduced binding as opposed to ~5% (seen with SIGN-

316 R.) The mutant $Arg^{A162}Ala$ bound DC-SIGN in a similar manner as it did to its homologue
317 SIGN-R showing a reduced binding of ~25% (Figure 4a).

318 **ghB substitution mutants bind differentially to DC-SIGN and SIGN-R**
319 Using ELISA, we examined the ability of DC-SIGN to bind to the ghB substitution mutants
320 $Arg^{B114}Gln$, $Arg^{B114}Ala$, $Arg^{B163}Glu$, $Arg^{B163}Ala$, $Arg^{B129}Ala$, $Arg^{B129}Glu$, $His^{B117}Asp$,
321 $Tyr^{B175}Leu$ and $Leu^{B136}Gly$ (29) . All the ghB substitution mutants bound DC-SIGN (Figure 4b)
322 and SIGN-R (Figure 5b) in a dose dependent manner. Substituting Arg^{B114} to Gln and Ala
323 resulted in a reduction of ~50% in the case of DC-SIGN (Figure 4b) and SIGN-R binding
324 (Figure 5b), suggesting that the Arg residue at this position plays an important role in the C1q-
325 DC-SIGN/SIGN-R interaction (Table 1, 2).
326
327 Substituting the ghB mutant Arg^{B129} with Glu and Ala caused a slight reduction of ~20% binding
328 with DC-SIGN (Figure 4b) and up to ~40% with SIGN-R (Figure 5b). When Arg^{B163} was
329 replaced with the negatively charged Glu, its affinity for DC-SIGN and SIGN-R was reduced by
330 50% and 35%, respectively (Figure 4b, 5b) (Table 1, 2); substitution with Ala resulted in 30%
331 reduction for DC-SIGN (Figure 4b) and increase by 20% for SIGN-R (Figure 5b) (Table 1.2). A
332 greater reduction in DC-SIGN and SIGN-R binding of ~60% was observed for the ghB mutant
333 His^{B117} substituted for Asp. For the ghB module, Tyr^{B175} substitution to Leu had the most
334 significant effect, showing a dramatic decrease of up to 90% in binding to DC-SIGN as well as
335 SIGN-R at a concentration of 0.625µg, this is not surprising due to its role in stabilizing the
336 gC1q domain.
337
338 **Residue Leu^{136} on ghB, important for IgG binding, is also involved in DC-SIGN binding**
339 Using a series of globular head single residue substitution mutants (29) , we sought to examine
340 which residues in the ghB chain offered complementary binding sites for DC-SIGN. Since
341 Leu^{B136} and Tyr^{B175} residues are considered important in maintaining the gC1q structure as well
342 as for IgG binding (32) , we used Leu^{B136} substituted for Glu and Tyr^{B175} substituted for Leu in
343 direct- binding ELISA. $Leu^{B136}Gly$ showed ~50% less binding to DC-SIGN at the highest
344 concentration (Figure 4b), suggesting that DC-SIGN and IgG binding sites on C1q (ghB) are
345 overlapping.

The contributions of ghC substitution mutants to DC-SIGN binding

The substitution mutants $His^{101}Ala$, $Arg^{156}Glu$ and $Leu^{170}Glu$ bound to DC-SIGN in a dose-dependent manner (Figure 4c). In fact, replacing Leu^{170} with Glu of the ghC chain reduced binding to DC-SIGN with a decrease in ~25% at the highest concentration. The ghC mutants $His^{101}Ala$ reduced binding by 10%, suggesting that the contributions of His^{101} and Leu^{170} are comparable in the DC-SIGN-C1q interaction. The ghC substitution mutants also bound to SIGN-R in a dose-dependent manner.

The mutants $His^{101}Ala$ appeared to show ~10% better binding to SIGN-R with compared to wild type, whereas $Leu^{170}Glu$ and $Arg^{156}Glu$ showed reduced binding by up to 25% at the highest concentration of 5 µg of SIGN-R (Figure 5c) (Table 2)

gC1qR and ghB compete for the same binding site on DC-SIGN

In view of the recent report of gC1qR, C1q and DC-SIGN forming a trimeric complex on immature DCs (15) , we examined whether DC-SIGN has complementary and overlapping binding site for C1q and gC1qR. We have recently mapped the gC1qR binding site on ghA, ghB and ghC (Pednekar et al, in revision). Since ghB was found to be the preferential binder of DC-SIGN, ghB modules were tested in a competitive assay. When different concentrations of ghB and a constant concentration of gC1qR were challenged against DC-SIGN, probing with anti-gC1qR polyclonal antibody revealed that with decreasing concentration of ghB, more gC1qR was able to bind to solid-phase DC-SIGN (Figure 6a), thereby implying an overlapping binding site between the proteins. 5µg of DC-SIGN and 5µg of gC1qR were able to bind efficiently, showing an OD of 1 (data not shown); this binding appeared to be drastically reduced when 5µg of gC1qR was allowed to compete with 5µg of ghB.

C1q, ghA, ghB, ghC inhibit the binding of DC-SIGN to gp120

C1q, ghA, ghB and ghC were able to inhibit the binding of DC-SIGN to immobilized gp120 in a dose dependent manner. The highest concentration of C1q, ghA, ghB, and ghC were able to significantly compete out the binding of DC-SIGN (Figure 6b).

The ghA, ghB, and ghC modules bind to cell surface expressed DC-SIGN

The binding of globular head modules to DC-SIGN was also performed using HEK 293 cells expressing DC-SIGN on the cell surface. The surface expression of DC-SIGN on DC-HEK cells was first confirmed with antibodies against DC-SIGN. To confirm the binding of individual globular head modules to DC-SIGN on DC-HEK cells, ghA, ghB and ghC fused with MBP were added to the DC-HEK cells (Figure 7). Incubation of the globular head modules and probing with anti-MBP monoclonal antibodies showed that each globular head module bound on the surface of DC-HEK cells co-localizing with DC-SIGN expressed on DC-HEK cells unlike MBP (Figure 7). This shows that globular heads interacted with DC-SIGN via head region not via MBP.

C1q inhibits DC-SIGN mediated transfer of HIV-1 to PBMC in culture

Since $CD4^+$ T cells and macrophages are the main cells targeted by HIV-1, we looked at the potential of C1q, ghA, ghB, ghC, and gC1qR to modulate DC-SING-mediated transfer of HIV-1 to activated PMBCs. As shown in Figure 8a, C1q considerably inhibited viral transfer to PBMCs in a dose-dependent manner on day 4 and 7. The globular head modules, ghA, ghB and ghC, surprisingly did not interfere with HIV-1 transfer, neither individually nor collectively, when compared to untreated or MBP-treated control, suggesting that the collagen region of C1q, and/or its multivalency of the gC1q domains are likely requirement for enforcing inhibitory properties. Addition of MBP in the Control wells did not significantly affect the p24 levels in comparison with untreated controls (data not shown). Furthermore, during the assay period, cellular viability was not affected by any of the protein treatments, suggesting that differences in the infectivity were not due to cell death.

We further examined the involvement of gC1qR with DC-SIGN in HIV-1 infection transmission (Figure 8b). gC1qR has previously been shown to inhibit CD4-gp120 interaction in HIV-1 isolates. It has also been recognized as a receptor on $CD4^+$ T cells that gp41 engages with in order to cause death of bystander $CD4^+$ T cells. We wanted to determine the role of gC1qR in DC-SIGN mediated infection with or without C1q. Figure 8b shows that gC1qR alone did not mediate viral transfer through DC-SIGN but significantly promoted viral transfer in the presence of C1q as well as the three globular head modules together for up to 7 days. This suggests that

the tripartite interaction between C1q, gC1qR and DC-SIGN enhances DC-SIGN mediated viral transfer. Thus, association of these proteins on DCs may actually promote HIV-1 infection. In addition, involvement of gC1qR in the tripartite complex is likely to negate the protective effect of C1q.

DISCUSSION

The role of complement in HIV-1 pathogenesis is well-documented. Complement-opsonized HIV-1 causes enhanced viral infection of $CD4^+$ T cell lines (33) , PBMCs, monocytes and macrophages (34) . DC-SIGN on the surface of immature DCs is involved in the capture of C3 opsonized R5 and X4 tropic HIV, and enhanced transmission to T cells (35) . C1q can bind to gp41 directly in an antibody-independent manner and activate the classical pathway (16) ; however, this leads to an enhanced infection of complement receptor bearing cells (36) . To escape complement mediated destruction, HIV-1 uses follicular DCs as a viral reservoir (37) , following its internalization via CR2, and remain within its protective recycling endosome. Following emergence from the endosome to the cell surface, HIV-1 infects follicular T cells through CD4. HIV-1 opsonized with C3b interacts with CR1 on erythrocytes with factor I dissociating erythrocytes from this complex and converting C3b to iC3b and C3d. C3d opsonized HIV-1 is then able to bind to CR2 on B cells (38) . In this study, we have examined involvement of C1q in a complement-independent interaction with HIV-1 via DC-SIGN.

C1q is a charge pattern recognition protein that binds to a variety of ligands via its gC1q domain (19) . Following its ability to interact with SIGN-R (31) , Hosszu et al recently have shown that C1q also recognizes a peptide derived from its homologue DC-SIGN (15) . In the current study, we made use of the availability of recombinant individual globular head modules of C1q (ghA, ghB and ghC) and its substitution mutants to establish that C1q binds DC-SIGN (and DC-SIGNR) via its gC1q domain.

Structure-function studies have demonstrated that the CRD region of DC-SIGN is the specific site for ligand binding and only functions in the presence of the neck region within the ECD (12) . We performed a series of binding experiments involving both the tetrameric forms of DC-SIGN and SIGN-R (comprising of the extracellular domain and CRD region) as well as the monomeric forms, which only consist of the CRD region. We asked the question whether DC-SIGN and

SIGN-R binding sites for C1q (and its gC1q domain) lies within their CRD region, or the α-helical neck region also plays an important role in these interactions. Both proteins have an increased affinity for glycoproteins containing high mannose oligosaccharides, such as mannan (39) , gp120 (10) and ICAM-3 (40) , via the CRD region. Here, individual globular head modules bound better to the tetrameric forms of DC-SIGN and SIGN-R as opposed to just the CRD region alone, indicating that the neck region, and hence, multimerization is needed to facilitate C1q binding. The neck region of DC-SIGN and SIGN-R interestingly differ most in their α helical structures (8) as the 23 amino acid repeats only show the first half of each repeat presenting a pattern of hydrophobic residues spaced at intervals, a feature that is abundant in most dimeric and trimeric coiled-coils (41) .

We also found that the ghB module bound better to DC-SIGN and SIGN-R. Previous studies have identified ghB as a key module of the gC1q domain in binding to IgG, PTX3 and CRP (42, 43) . Interestingly, C1q and ghB bound SIGN-R better than DC-SIGN, given 77% sequence similarity. We also expressed and purified globular head mutants (29) where single amino acid residues were substituted in order to localize key residues involved in C1q interaction with its various ligands. These mutants were designed based on the crystal structure and are the residues known to be important in binding to various C1q ligands (42) . It appears that IgG and DC-SIGN binding sites on ghB are overlapping and shared since Lys^{136} and Tyr^{175} on ghB, which have been previously shown to be important for binding to IgG (29) and for gC1q assembly (44) . We also examined the roles of Arg^{114}, Arg^{129} and Arg^{163} of the ghB module since Arginine residues have previously been shown to be important for the C1q-IgG interaction (45) . Moreover, Hosszu et al have reported that C1q binds DC-SIGN via its IgG binding site. Our results highlighted the significance of Arg^{114} in C1q interaction with DC-SIGN and SIGN-R (Figure 4b, 5b). Substituting Arg^{114} with the polar residue Glutamine and hydrophobic residue Ala led to ~80% reduction, highlighting a very important role for $Arginine^{114}$ of B chain in C1q-DC-SIGN interaction. In addition, Tyr^{175} appears critical for C1q interaction with DC-SIGN and SIGN-R (Figure 4b, 5b); the binding analysis revealed a dramatic reduction (82% for DC-SIGN and 90% for SIGN-R following substitution of Tyr with Leu. This is not the first time Tyr^{175} has been shown to be a critical residue in gC1q binding (32) . Gadjeva et al have shown that this residue mainly constitutes C1q binding to IgM. Overall, our binding studies suggest that Tyr^{175} ^{and} Arg^{114} of ghB are critical for the C1q-DC-SIGN and C1q-SIGN-R.

The known dual roles of DC-SIGN as a facilitator of adaptive immune response as well as promoter of HIV-1 infection prompted us to examine if innate immune soluble factors such as C1q and gC1qR can potentially modulate viral transmission via DC-SIGN (46) , similar to reports involving $CD4^+$ T cells (47) and a lectin drug GRFT (*Griffithsia)* isolated from the red algae (48) . We also included DC-SIGN-R (DC-SIGN-Related), a homolog of DC-SIGN, in our study. DC-SIGN-R is expressed on endothelium including liver sinusoidal (49) , lymph node sinuses and placental capillary (8) . DC-SIGN-R can bind ICAM-3 as well as gp120 to facilitate HIV-1 viral infection (49) . As a receptor for bacterial dextrans (50) and capsular pneumococcal polysaccharide (CPS) of *S. pneumoniae,* DC-SIGN-R can cause proteolysis of C3 (31) . DC-SIGN-R is shown to be highly expressed by spleen marginal zone macrophages (MZM) and lymph node macrophages (51). SIGN-R1 in MZM interacts with C1q in the spleen and enhances apoptotic cell clearance via activation of the classical pathway (52) .

The transmembrane envelope gp41 protein of HIV-1 is known to interact with C1q (53) through its A chain (19) , leading to complement activation but no viral lysis (54) . Instead, the virus is transmitted to complement receptor bearing cells such as macrophages and $CD4^+$ T cells allowing infection to take place (36, 53, 55, 56) . HIV-1 infected $CD4^+$ T cells can activate the classical pathway via shedding of gp120, leading to unmasking of the gp41 epitope 601-613 available for interaction with C1q (23) . C1q is also involved in a range of processes independent to its complement functions (57, 58) , including DC differentiation (59) . C1q, along with its globular head receptor gC1qR and DC-SIGN can co-localize on the surface of blood precursor DCs to promote DC differentiation (15) . gC1qR, a multi-functional pathogen recognition receptor (60, 61) , can also interact with gp41 of HIV-1 (17) on uninfected $CD4^+$ T cells and upregulate NK cell ligand NKP44-L, rendering healthy $CD4^+$ T cells susceptible to NK cell lysis. Since DC-SIGN is a receptor for HIV-1 through its binding to gp120, it is interesting that it co-localizes with C1q and gC1qR, the two proteins, also known for HIV-1 binding and transmission of the viral infection. Such association forming a tri-molecular unit on the target cell surface may create a vehicle that promotes pathogen entry and immunosuppression (15) .

We wanted to examine if C1q-DC-SIGN interaction modulated HIV-1 transfer. We found that full length C1q, but not its individual globular heads, suppressed DC-SIGN-mediated HIV-1 transfer to activated PBMCs. Curiously, addition of gC1qR negated the protective effects of C1q

by enhancing DC-SIGN-mediated viral transfer. gC1qR, as an inhibitor of HIV-1 infection, can block the interaction between CD4 and gp120 and prevent viral entry (24) . Since DC-SIGN binds to gp120 and gC1qR to gp41, both processes which are shown to promote infection, we can consider that even if gC1qR does interfere with the DC-SIGN-gp120 interaction, its active binding site for gp41 is still available to facilitate infection. The increased viral transmission of gC1qR seen when in association with C1q suggests that C1q bound to gC1qR can enhance its function. It is possible that C1q plays a protective role by blocking access of gp120 to DC-SIGN (Figure 9). This can happen if C1q shares sharing binding sites on DC-SIGN. The globular heads, individually or in combination, did not appear to inhibit virus transmission as full length C1q, suggesting that the Collagen domain of C1q, and/or probably oligomeric form of C1q is required for the observed inhibitory effect.

In summary, we found that gC1qR can alter C1q-DC-SIGN interaction in a way that it promotes viral transfer, thus neutralizing the protective effect of C1q. The tripartite complex involving DC-SIGN-gC1qR-C1q probably leads to an increase in the distance between DC-SIGN and C1q that permits DC-SIGN interaction with gp120; this allows DC-SIGN and gC1qR to bind to the virus with enhanced affinity (Figure 9).

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Table 1

Bindings of globular head modules and its mutants with DC-SIGN (% binding ± SD)	
ghA	100.00 ±0.0
R162A	77.71 ±8.85
R162E	66.41 ±2.38
ghB	100.0 ± 23.62
R129E	93.91 ± 8.34
R129A	75.05 ± 7.22
R163A	68.76 ± 12.78
R114A	57.37 ± 4.45
R114Q	52.46 ± 1.94
R163E	48.33 ± 8.89
L136G	47.74 ± 9.17
T175L	37.92 ± 7.50
H117D	36.35 ± 10.28
ghC	100.00±0.00
R156E	101.41±0.27
H101A	90.20±2.27
L170E	78.32±1.47

Table 2

Bindings of globular head modules and its mutants with SIGN-R (% binding ± SD)	
ghA	100.00 ±0.00
R162E	94.78 ±15.08
R162A	73.17 ±9.54
ghB	100.00 ± 0.00
R129E	136.70 ± 21.82
R163A	119.68 ± 30.09
R129A	92.55 ± 2.26
R163E	65.69 ± 4.89
R114A	61.44 ± 0.38
L136G	53.99 ± 1.88
R114Q	50.27 ± 3.39
H117D	40.96 ± 2.26
T175L	37.23 ± 1.50
ghC	100.00±0.00
H101A	112.60±2.86
L170E	79.81±20.40
R156E	75.77±4.62

FIGURE LEGENDS

Figure 1: SDS-PAGE under reducing conditions (12% v/v) showing purified fractions of soluble DC-SIGN tetramer (a), DC-SIGN Monomer (b), SIGN-R tetramer (c), and SIGN-R monomer (d), following purification by Mannose-Agarose affinity chromatography.

Figure 2. Interaction of C1q, ghA, ghB and ghC with DC-SIGN tetramer and monomer. (a) Microtitre wells coated with different concentrations (5, 2.5, 1.25, 0.625 µg/well) of DC-SIGN Tetramer or Monomer were probed with 2 µg/well of C1q. Bound C1q was detected with anti-C1q polyclonal antibodies (1:1000 in PBS) and Protein A HRP conjugate (1:1000 in PBS). BSA was used as a negative control protein. (b) Binding of ghA, ghB and ghC to DC-SIGN Tetramer and DC-SIGN Monomer involved coating a range of concentrations of the respective proteins on microtitre wells, which were then incubated with a fixed concentration of ghA, ghB, ghC and MBP (2.5 µg/well in 5mM $CaCl_2$ buffer) at 37ºC. Binding was detected using anti-MBP monoclonal antibodies (1:5000 in PBS) and then rabbit anti-mouse IgG-HRP (1:5000 in PBS). (d) Far western blot to show DC-SIGN tetramer binding to membrane-bound ghA, ghB and ghC: 15µg of ghA, ghB and ghC (BSA and MBP as negative control proteins) were run on a 12% SDS-PAGE gel, and then transferred on to nitrocellulose membrane. The blot was incubated with 50µg of DC-SIGN in PBS overnight at room temperature. The bound DC-SIGN protein was detected using anti-DC-SIGN polyclonal antibodies and Protein A HRP conjugate. Bands were developed using DAB tablets dissolved in water.

Figure 3: Interaction of C1q, ghA, ghB and ghC with SIGN-R tetramer and monomer. (a) ELISA to examine binding of C1q to SIGN-R Tetramer and SIGN-R Monomer: SIGN-R Tetramer or Monomer were coated at different concentrations, followed by addition of 2µg/well of C1q. Bound C1q was probed with anti-C1q polyclonal antibodies (1:1000 in PBS) and Protein A HRP (1:1000 in PBS), and the colour was developed using OPD. (b) Binding of ghA, ghB and ghC to SIGN-R Tetramer and (c) SIGN-R Monomer**:** Different concentrations of SIGN-R Tetramer (b) and SIGN-R Monomer (b) were coated on microtitre wells in carbonate buffer and incubated overnight at 4ºC and then incubated with ghA, ghB, ghC and MBP (2.5µg/well in 5mM $CaCl_2$ buffer). Binding was detected using anti-MBP monoclonal antibody and rabbit anti-mouse IgG-HRP conjugate.

Figure 4: Binding of globular head substitution mutants to DC-SIGN: Different concentrations of DC-SIGN tetramer were coated on microtitre wells in carbonate buffer overnight at 4ºC. Wells were then incubated with 2.5µg/well (in $CaCl_2$) of recombinant globular head wild type and mutant proteins and probed with anti-MBP monoclonal antibody and rabbit anti-mouse IgG-HRP conjugate, as described earlier. Per cent binding was calculated for each mutant using binding of the wild type globular head module as 100%. (a**)** Binding of ghA, ghA-R162E and ghA-R162A to DC-SIGN; (b) Binding of ghB mutants ghB- L136G, ghB-T175L, ghB- R114Q, ghB-R114A, ghB-R163A, ghB-R163E, ghB-R129E, ghB-R129A and ghB-H117D to DC-SIGN; (c) Binding of ghC mutants ghC-R156E, ghC-L170E and ghC-H101A to DC-SIGN.

Figure 5: Binding of globular head substitution mutants to SIGN-R: Different concentrations of SIGN-R tetramer were coated on microtitre wells in carbonate buffer overnight at 4ºC and then incubated with 2.5µg/well of recombinant globular head wild type and mutant proteins and probed with anti-MBP monoclonal antibody and rabbit anti-mouse IgG-HRP conjugate. % binding was calculated for each mutant using binding of the wild type globular head module as 100%. (a) Binding of ghA, ghA-R162E and ghA-R162A to SIGN-R; (b) Binding of ghB mutants ghB- L136G, ghB-T175L, ghB- R114Q, ghB-R114A, ghB-R163A, ghB-R163E, ghB-R129E, ghB-R129A and ghB-H117D to SIGN-R; (c) Binding of ghC mutants ghC-R156E, ghC-L170E and ghC-H101A to SIGN-R.

Figure 6: Competitive ability of ghA, ghB and ghC for interaction with DC-SIGN in the presence of gC1qR and gp120. (a) ELISA to assess whether gC1qR and ghB directly compete for the same binding site on DC-SIGN**:** DC-SIGN was coated at 5µg/well overnight at 4°C. Wells were blocked with 2% BSA in PBS for 2 hours at 37º. 5µg/well of gC1qR and different concentrations of ghB (5, 2.5, 1.25, 0.625µg/well) were added in buffer containing 5mM $CaCl_2$. Incubation was carried out at 37º for 1.5 hours and 4º for 1.5 hours. Following repeated washes, bound gC1qR was probed using rabbit anti-gC1qR polyclonal antibodies (1:1000) and Protein A-HRP (1:1000). Colour was developed using OPD substrate; (b) Competition between DC-SIGN tetramer and C1q globular head modules to bind solid-phase gp120. Microtitre wells were coated with 250 ng of gp120. Various concentrations of ghA, ghB, ghC, and C1q and constant 2.5ug/ml of DC-SIGN were incubated at 37°C for 1 h and then at 4°C for 1 h. The binding of DC-SIGN to gp120 in the presence of globular heads or C1q was detected using rabbit anti-DC antibody (1:500), probed with PA HRP (1:5000). 100% binding was taken of DC-SIGN alone binding to gp120.

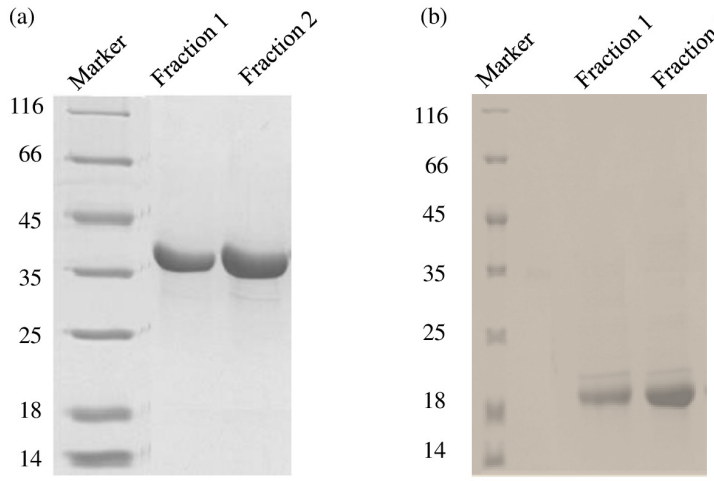
Fig 7: *In vitro* binding of Globular heads modules to DC-SIGN expressed on HEK cells: DC-SIGN expressing HEK cells (DC-SIGN cells) were incubated with recombinant globular head modules (ghA, ghB, ghC) and MBP as control for 30 mins at 37°C. DC-HEK cells were fixed with 4% PFA, washed and blocked with 5% FCS, and probed with mouse anti MBP antibody to detect the presence of MBP fused globular head modules and rabbit anti DC-SIGN to detect DC-SIGN expressed on the cells. Goat anti-mouse secondary antibody conjugated with Alexa Fluor 568 was used to detect binding of globular heads to DC-SIGN visualized using goat anti-rabbit IgG conjugated with Alexa Fluor 488 antibody. Scale bar 20µm.

Figure 8: HIV transfer assay mediated by DC-SIGN

HEK/DC-SIGN cells were grown in a 12 well-plate to form a confluent layer. Different concentrations of proteins were added to the cells and incubated for 2 hours for binding. Unbound proteins were removed and cells were challenged with 2.5ng/ml p24 of HIV-1 (SF-2 strain) for 1 hour. Unbound virus was washed off and cells were co-cultured with PHA-activated PBMCs for 24 hours. PBMCs were separated from the DC-HEK monolayer and cultured separately for 7 days to determine viral titre of the supernatants collected on day 4 and day 7. (a) C1q, ghA, ghB, ghC and ghABC; (b) gC1qR in presence of C1q, ghA, ghB, ghC and ghABC. Data represents Mean ± SD. $P<0.05$ is considered significant. * and # indicate statistical significance in comparison untreated controls of day 4 and day 7, respectively.

Figure 9. Diagrammatic model explaining the possible implications of the tripartite molecular interplay between DC-SIGN, C1q and gC1qR. (a) By virtue of its ability to bind to DC-SIGN on the cell surface, C1q is likely to inhibit interaction between DC-SIGN and HIV-1 gp120, resulting in the inhibition of viral transfer. (b) On the DC/Monocyte surface, a tri-molecular receptor complex is formed between gC1qR, C1q and DC-SIGN. Although each of these molecules can bind the HIV-1 virus independently, we postulate that it is the binding of the HIV-1 gp-41 to both gC1qR and C1q that initiates the membrane fusion before the final binding of gp120 to DC-SIGN and/or CD4, eventually allowing the internalization of the virus. It is possible that HIV-1 interaction with DC/monocytes causes recruitment of gC1qR to the cell surface, or its secretion, which in turn, can bind to C1q globular heads, thereby neutralizing the protection offered by C1q.

DC-SIGN Tetramer **DC-SIGN Monomer**



SIGN-R Tetramer **SIGN-R Monomer**

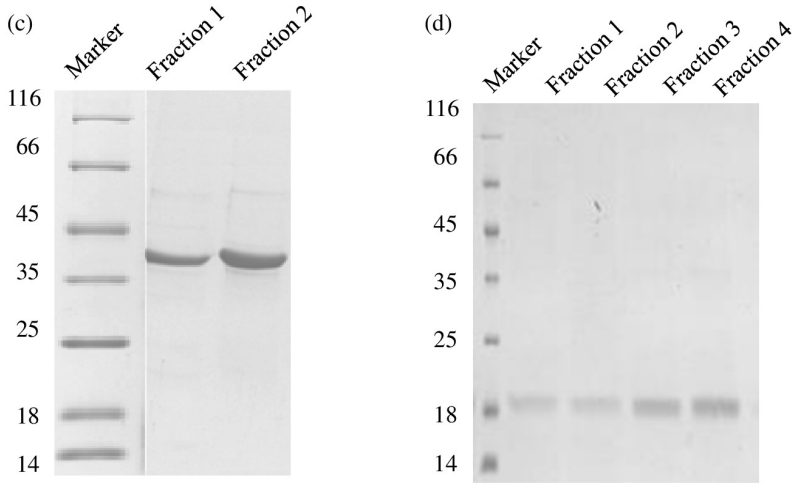


Figure 1 Pednekar et al.

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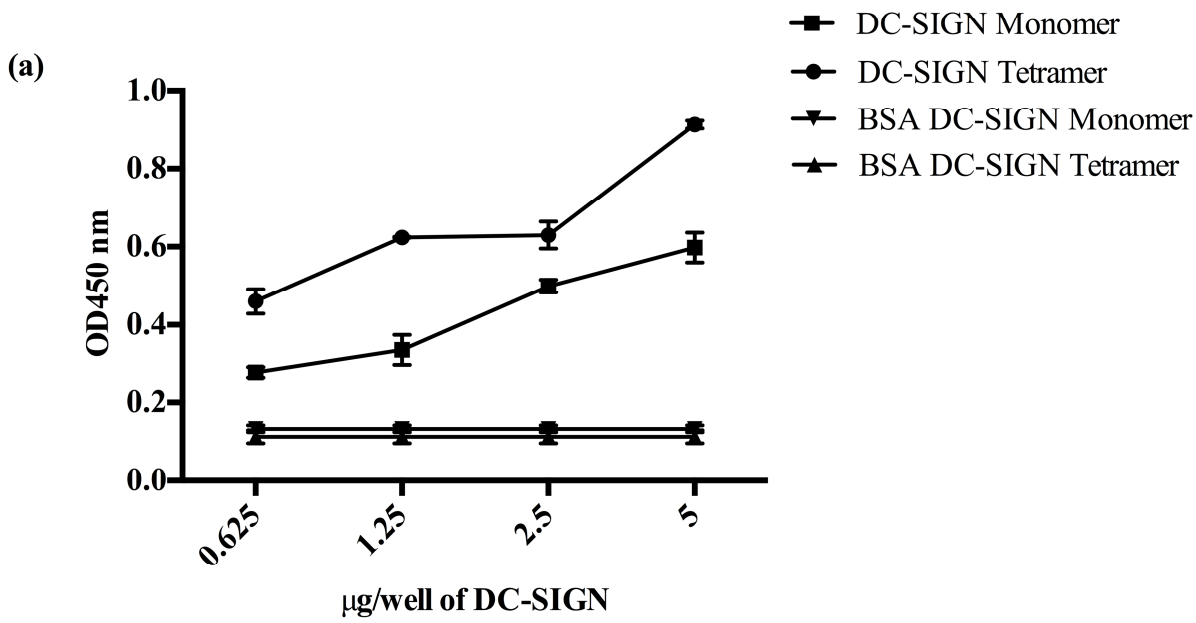

Figure 2: Pednekar et al

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Binding of C1q globular heads modules to tetrameric DC-SIGN

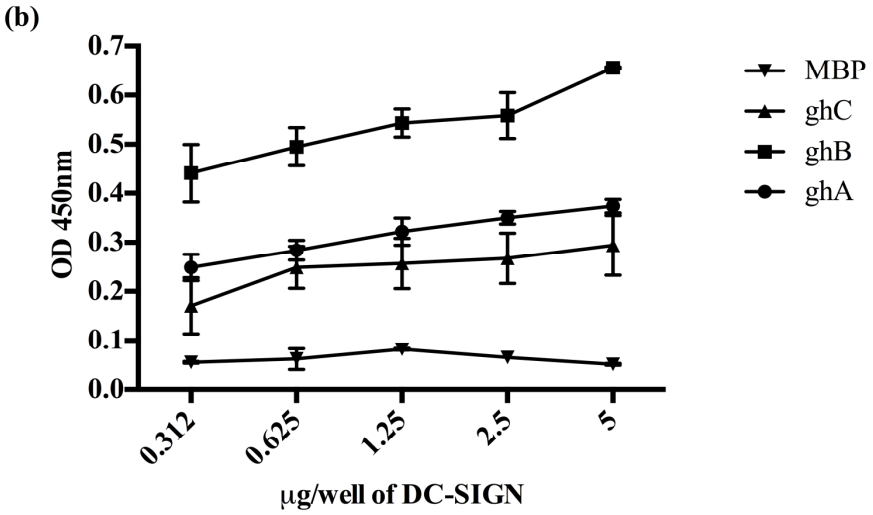

Figure 2: Pednekar et al

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Binding of C1q globular heads modules to monomeric DC-SIGN

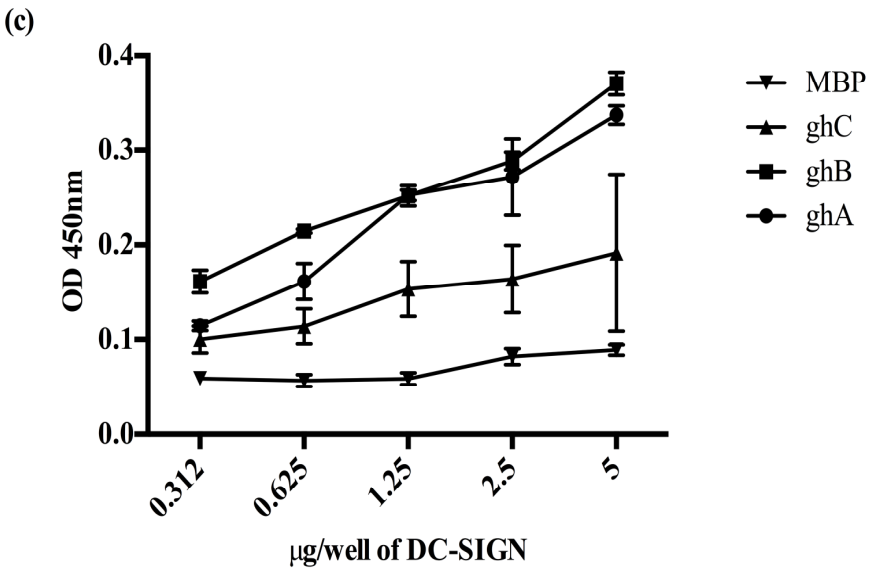

Figure 2: Pednekar et al

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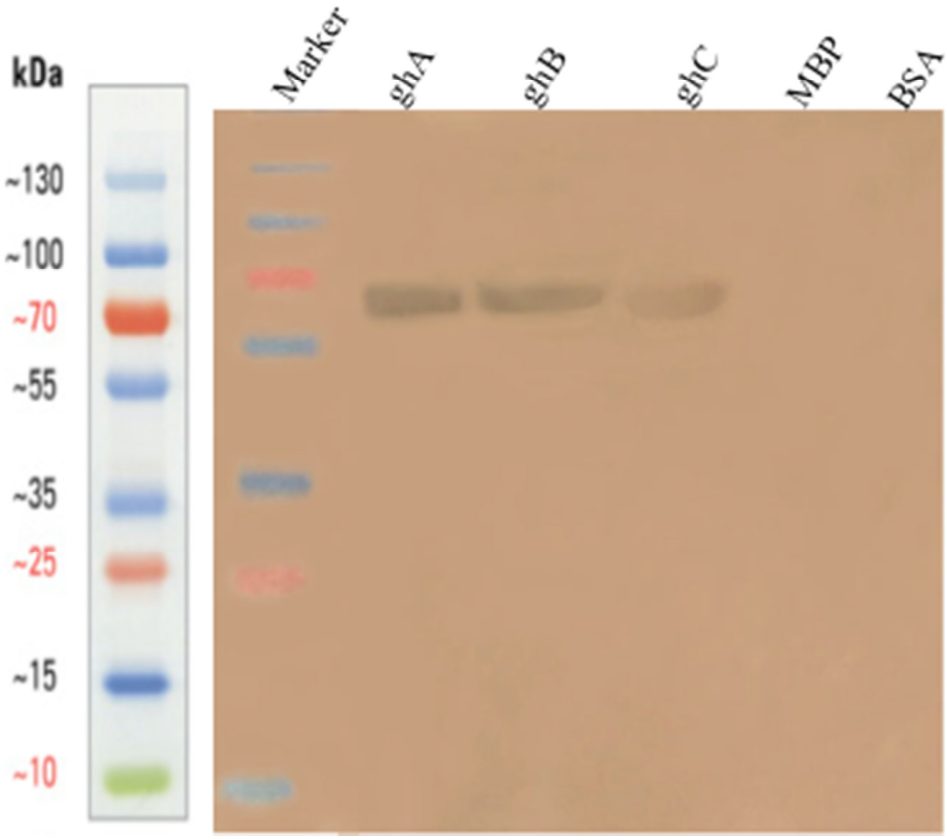
(d) Far Western Blot showing immobilized ghA, ghB and ghC bind soluble DC-SIGN tetramer



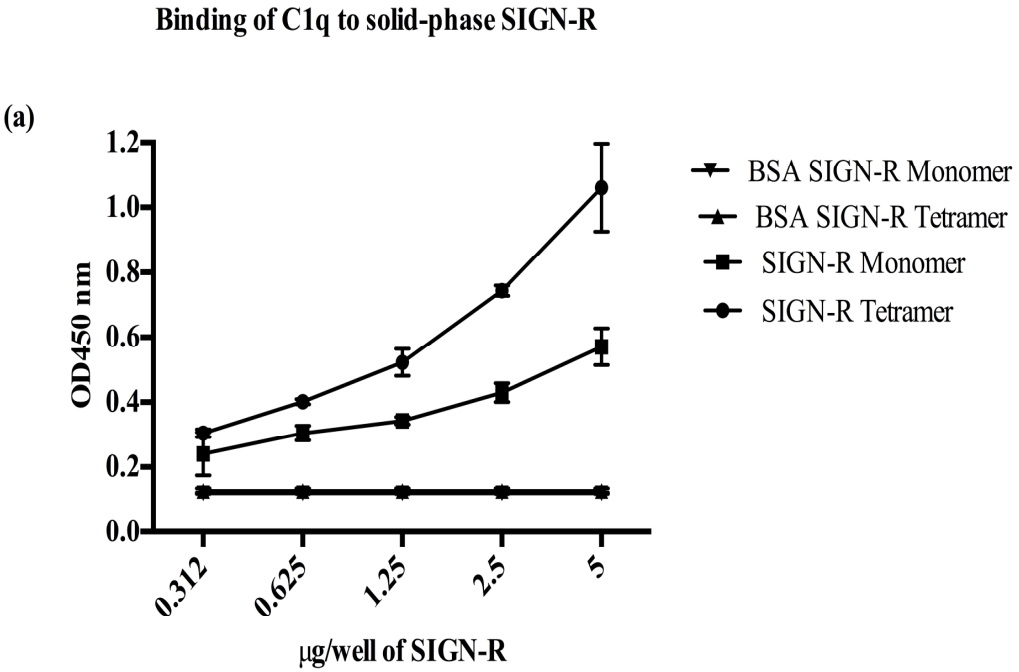


Figure 3: Pednekar et al

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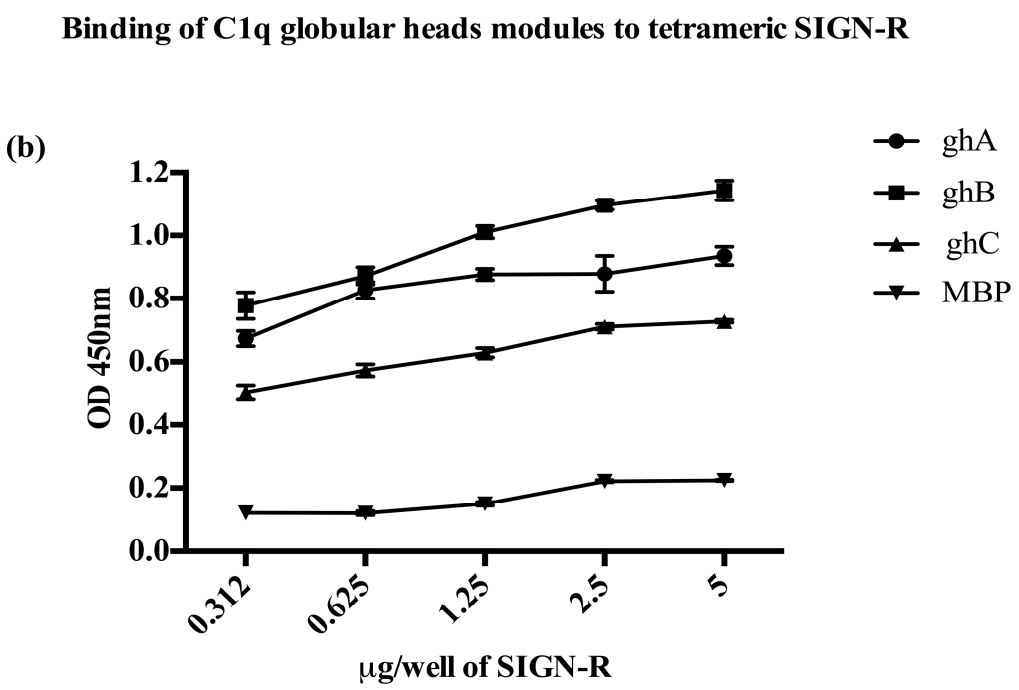

Figure 3: Pednekar et al

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Binding of C1q globular heads modules to monomeric SIGN-R

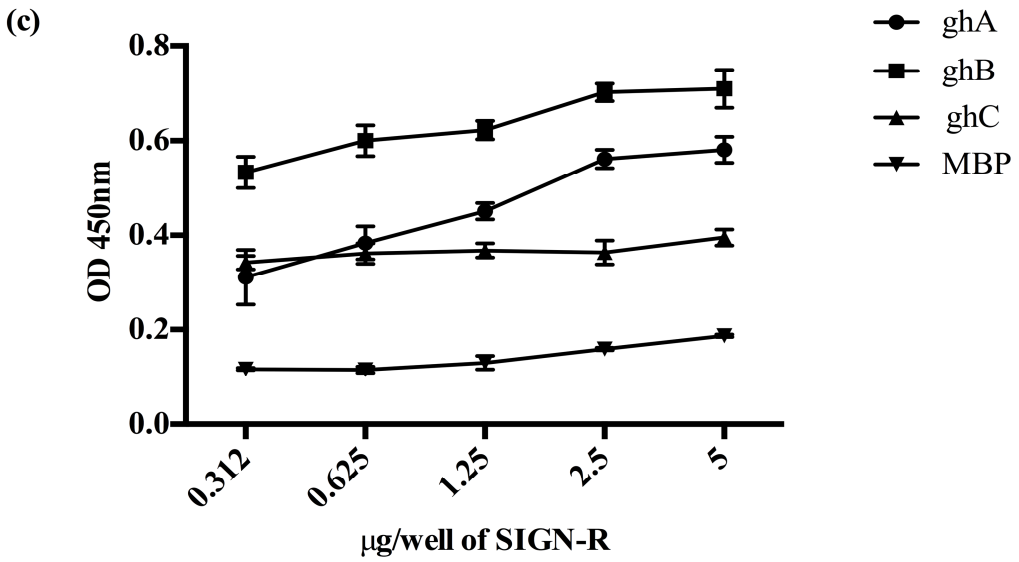

Figure 3: Pednekar et al

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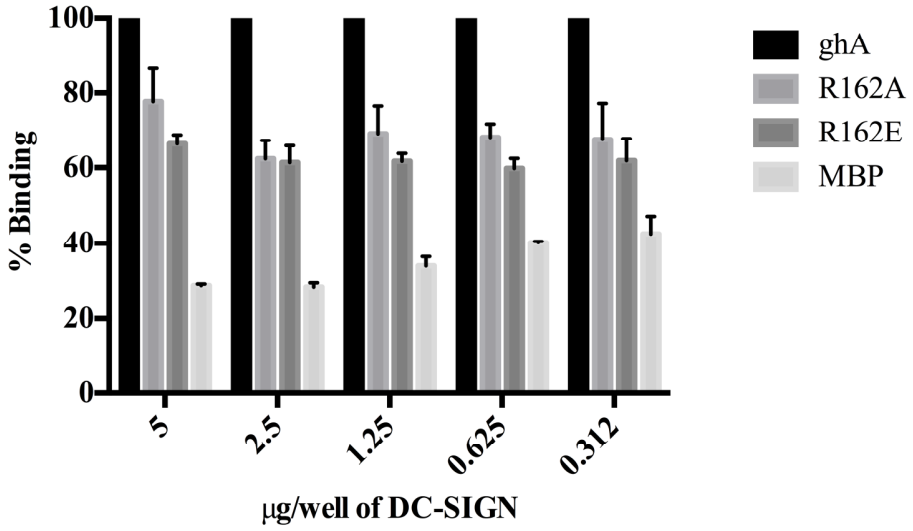

Figure 4: Pednekar et al

(b)

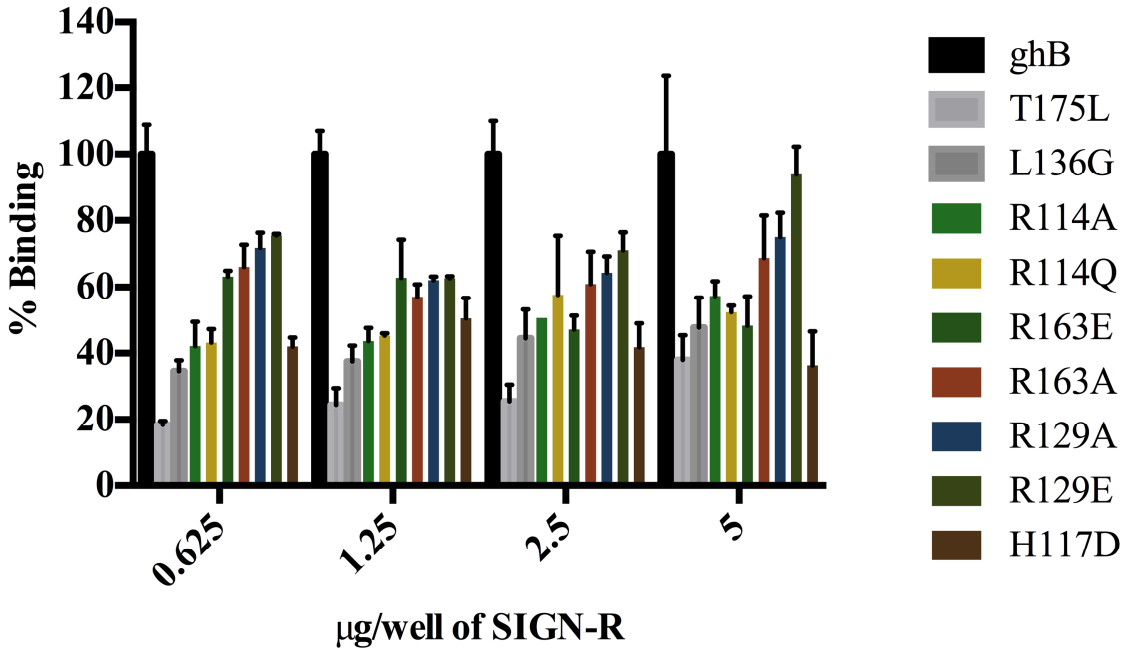

Figure 4: Pednekar et al

(c)

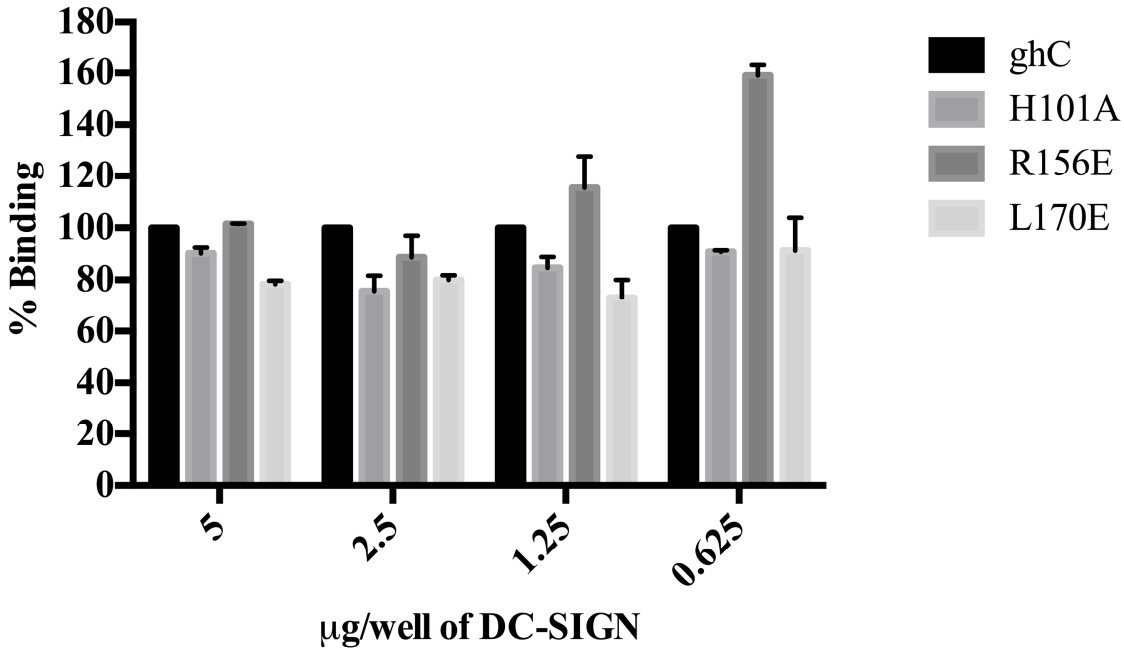

Figure 4: Pednekar et al

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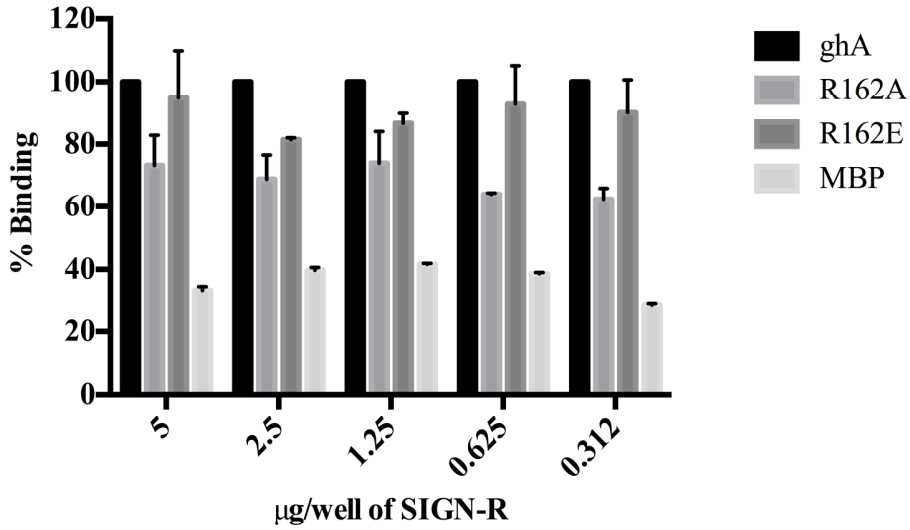

Figure 5: Pednekar et al

(b)

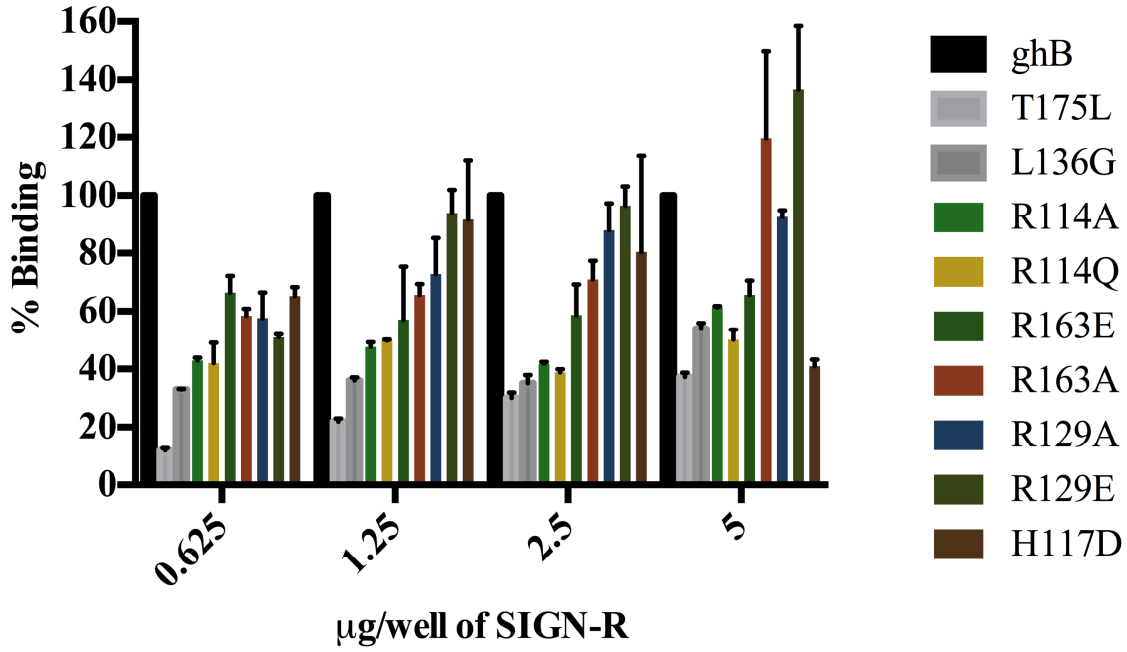

Figure 5: Pednekar et al

(c)

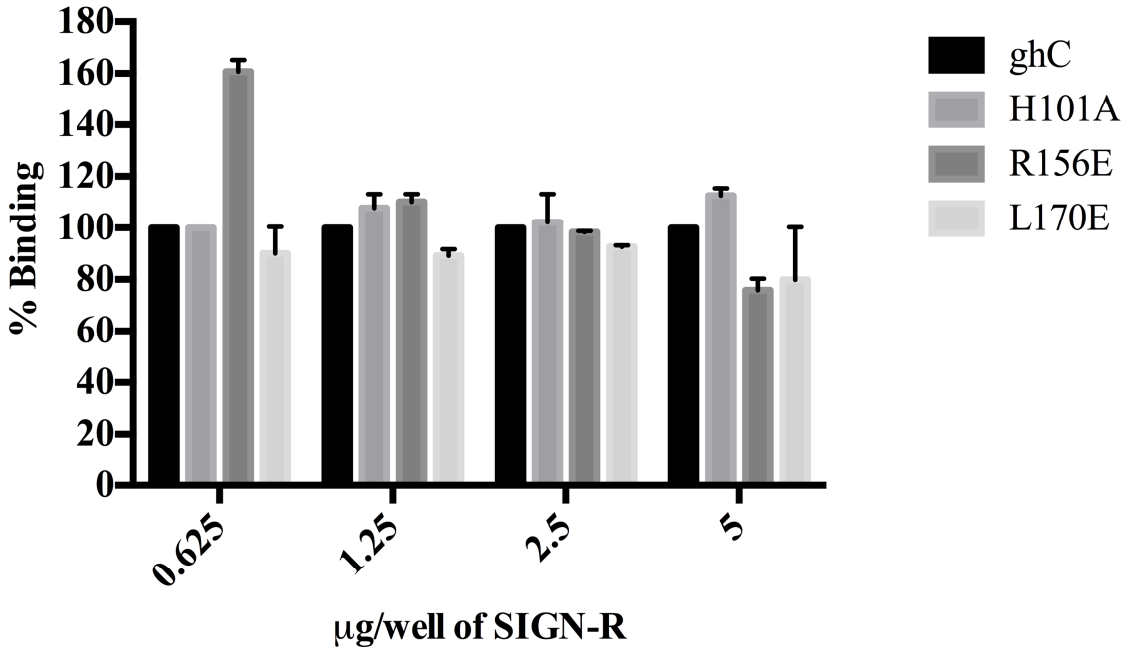

Figure 5: Pednekar et al

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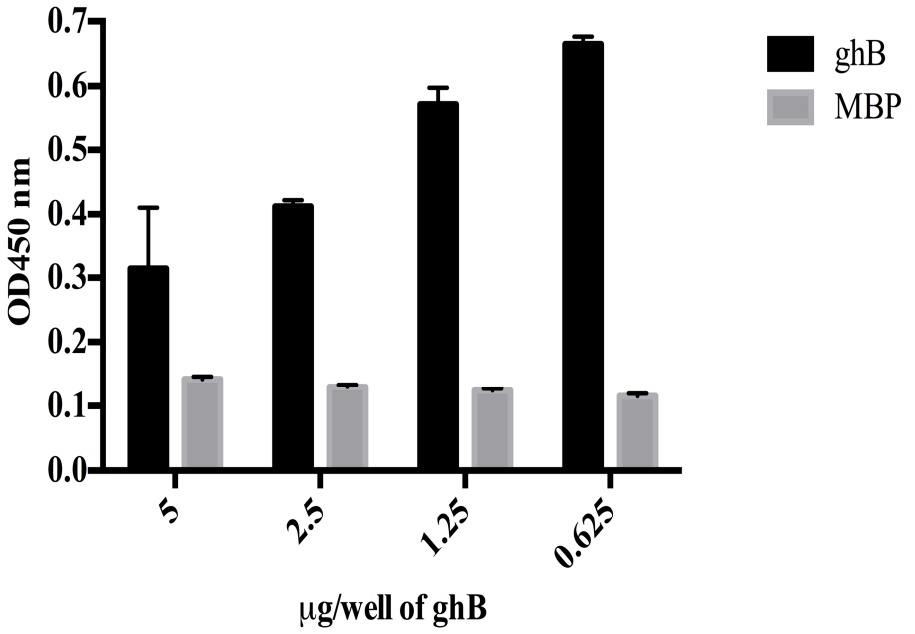

Figure 6: Pednekar et al

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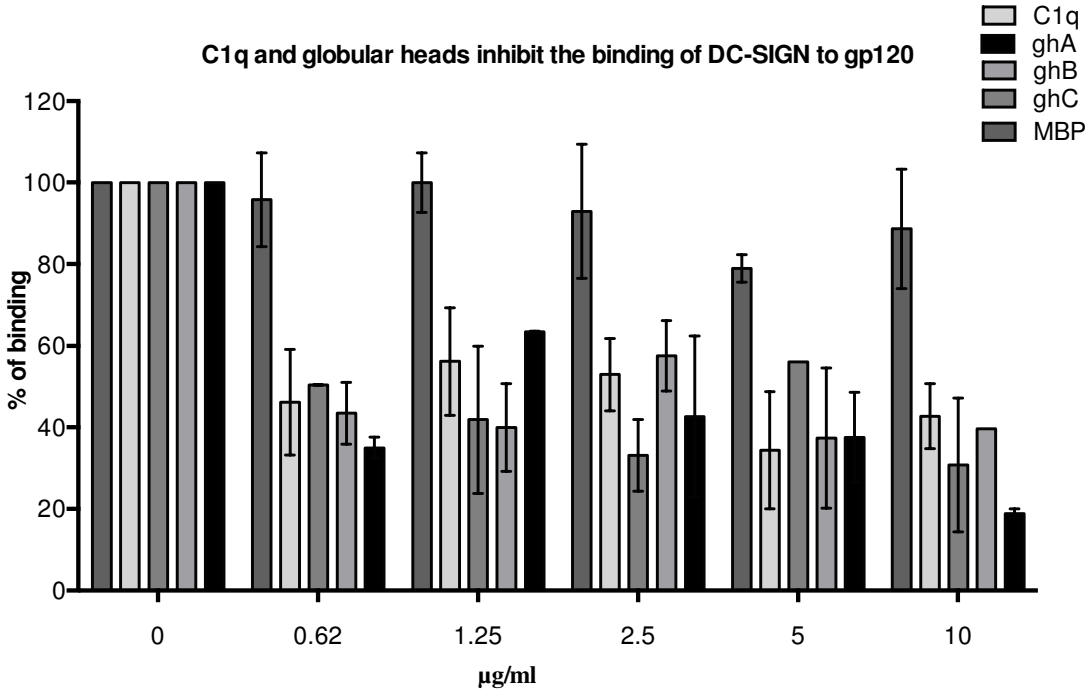

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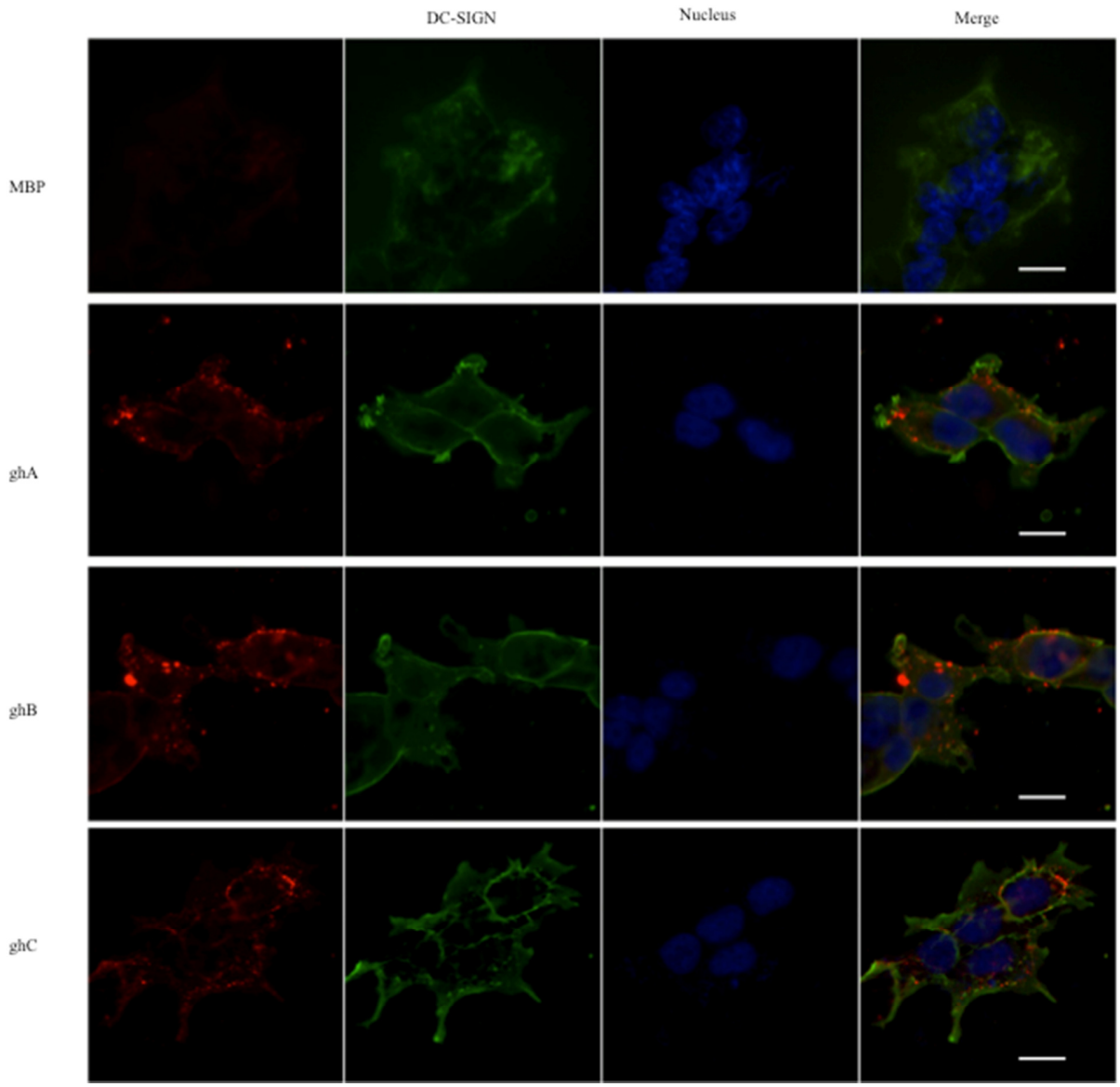
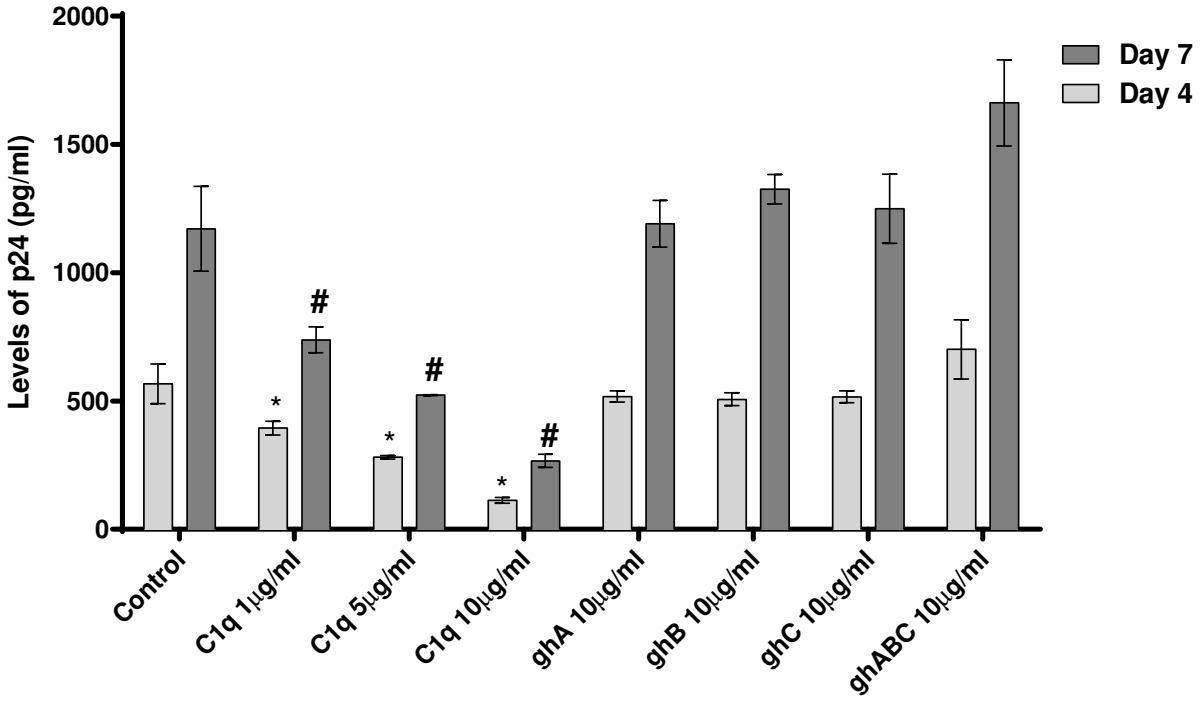


Figure 7.

Figure 8 (a)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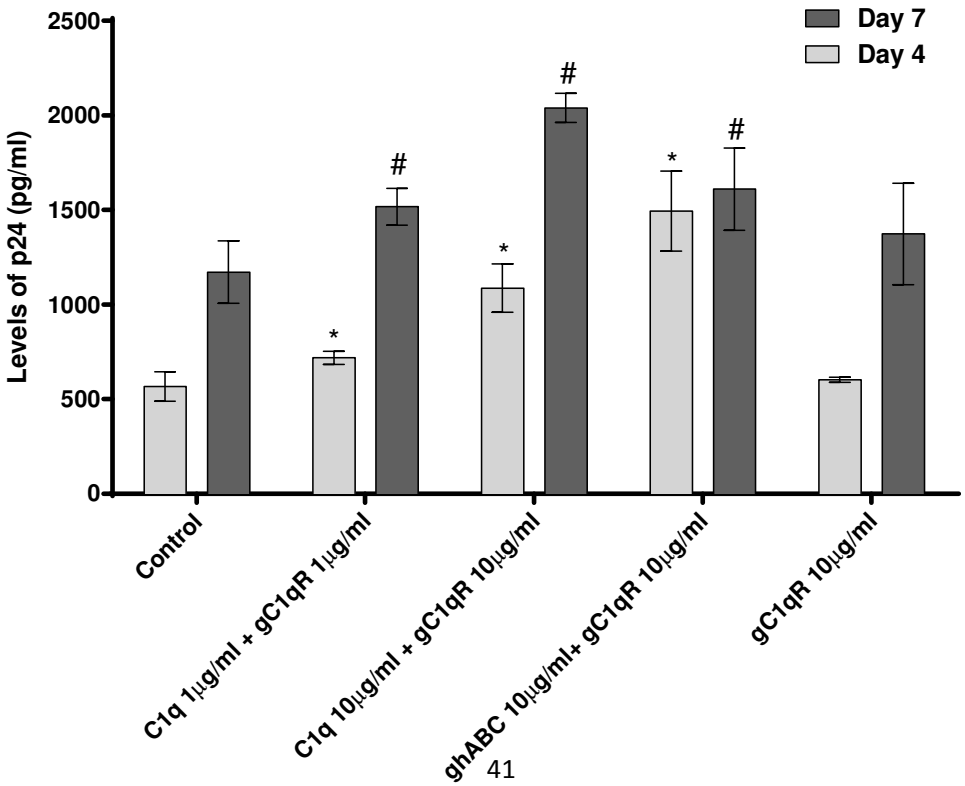
ghABC 10μg/ml

(b)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C1q 10μg/ml + gC1qR 10μg/ml
ghABC 10μg/ml+ gC1qR 10μg/ml
gC1qR 10μg/ml

Figure 9.